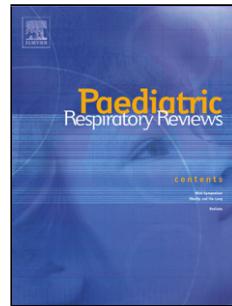


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Middle East Respiratory Syndrome Coronavirus “MERS-CoV”:

Current Knowledge Gaps

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EDUCATIONAL AIMS:

The reader will be able to:

- Get the latest update on MERS-CoV, including its clinical features, diagnosis and management.
- Learn about the adult and paediatric aspects of MERS-CoV.
- Understand the gaps in current knowledge and future research in the field of MERS-CoV.

SUMMARY

The Middle East respiratory syndrome coronavirus (MERS-CoV) that causes a severe lower respiratory tract infection in humans is now considered a pandemic threat to the Gulf region. Since its discovery in 2012, MERS-CoV has reached 23 countries affecting over 1000 people, including a dozen children, and claiming over 400 lives. Compared to SARS (severe acute respiratory syndrome), MERS-CoV appears to kill more people (40% versus 10%), more quickly, and is especially more severe in those with pre-existing medical conditions. Most MERS-CoV cases (>85%) reported thus far have a history of residence in, or travel to the Middle East. The current epidemiology is characterised by slow and sustained transmission with occasional sparks. The dromedary camel is the intermediate host of MERS-CoV, but the transmission cycle is not fully understood. In this current review, we have briefly summarised the latest information on the epidemiology, clinical features, diagnosis, treatment and prevention of MERS-CoV especially highlighting the knowledge gaps in its transmission dynamics, diagnosis and preventive strategy.

INTRODUCTION

The Middle East respiratory syndrome coronavirus (MERS-CoV) was first isolated by Zaki and co-workers in June 2012 from a Saudi male aged 60 years who died of severe pneumonia and renal failure. [1] Currently, the virus has affected 1082 individuals in 23 countries across the globe, claiming 439 (40%) lives, and thus poses a public health challenge to the Arabian Peninsula and elsewhere (Figure 1). [2, 3] MERS-CoV outbreak began a decade after the epidemic of the severe acute respiratory syndrome (SARS) that caused a global scare in 2002-2003 affecting over 8000 people across the world killing one tenth of them. [4-6] However, unlike SARS, which disappeared within a year, the MERS-CoV epidemic continues (Table 1).

Since its discovery, a number of excellent reviews have been published describing the important aspects of MERS-CoV, but few have summed up the current research gaps in light of the spectrum of burning questions ranging from its clinical presentation to molecular virology. [7-20] In this review, we have summarised the latest findings in the field of MERS-CoV, especially its molecular virology, epidemiology, clinical features, diagnosis and infection control management plans, and highlighted the knowledge gaps in transmission modes, and attempted to explain unanswered questions raised by other investigators.

Literature searches were performed in PubMed, Promed Mail, Google and Google Scholar on articles published between September 2012 and March 2015. The following vocabulary terms were used: 'coronavirus', 'mers-cov', 'coronavirus mers-cov', 'mers-cov review', 'mers-cov infection', 'mers-cov outbreak', 'mers-cov case studies/reports', 'mers-cov in camel', 'mers-cov in bat', 'mers-cov transmission', 'mers-cov pathogenesis', 'mers-cov

antibodies', 'mers-cov receptor', 'mers-cov in animal', 'mers-cov diagnosis', 'mers-cov treatment', 'mers-cov vaccine', 'mers-cov in saudi' and 'mers-cov in health care worker'.

VIROLOGY OF MERS-COV

MERS-CoV is a positive-sense, enveloped, single-stranded RNA virus belonging to the genus of *Betacoronavirus* within the subfamily *Coronavirinae*. [21] Known in the begining variously as novel corona virus (nCoV), London1_novel CoV 2012, MERS-CoV has become the first lineage of *Betacoronavirus* known to infect humans. [7, 9,] Having 90% sequence homology, MERS-CoV is more closely related to bat coronaviruses HKU4 and HKU5 (lineage 2C) than it is to SARS-CoV (lineage 2B), [4, 21] and is genetically related to other *Betacoronaviruses* isolated from bats in Europe, Mexico, Africa, Hong Kong and China. [22-24]

Following its first isolation, the virus was propagated in African green monkey and rhesus macaque kidney cells (Vero and LLC-MK2 cell lines, respectively), [1, 25] and subsequently, in other cell lines including human derived cells like Calu-3, HFL, Caco-2, Huh-7, HEK, and His-1. [25] MERS-CoV propagates well in human bronchial and lung tissues. [26] Cell lines derived from camels and goats were more functional for the replication of MERS-CoV than those from other species including dogs, cats, mice, hamsters, ferrets and equids [13, 27-29], indicating that the binding site of MERS-CoV could vary by species. [27, 30]

Hemida et al. (2014) described the full genome of MERS-CoV from dromedaries demonstrating that it had 99.9% homology with human clade B MERS-CoV, [31] which was confirmed by a subsequent study. [13] MERS-CoV has also high sequence homology (94%) with coronavirus carried by *Pipistrellus* bats. [32]

Electron microscopy reveals that the virion contains club-like projections, representing viral spike peplomers, emanating from the viral membrane. MERS-CoV gains entry into target cells by binding to the receptor binding domain (RBD) on its spike glycoprotein to human receptor dipeptidyl peptidase 4 (hDPP4), whereas SARS enters the target cells via angiotensin converting enzyme 2 (ACE2). [25, 33, 34] Both ACE2 and hDPP4 are commonly expressed in some human organs such as kidneys. [33] MERS-CoV utilises host proteases to gain entry into lung cells. [35] The spike protein on the viral envelope is activated by a protease, called furin, and mediates membrane fusion which eventually supports the virus entry into a host cell. [33, 36, 37] Consequently, protease inhibitors (e.g., camostat) are found to block MERS-CoV entry into cells. [35]

MERS-CoV inoculated into rhesus macaques caused a transient lower respiratory tract infection, a multifocal, mild to marked interstitial pneumonia, with virus replication occurring mainly in alveolar pneumocytes, [38] and caused extensive lethal pneumonia in the common marmoset. [39] *In vitro* experiments suggest that unlike SARS, MERS-CoV has cytopathic effects on kidney cells, and compared to SARS, MERS patients are more likely to develop acute renal failure. [33]

Detailed pathogenesis of MERS-CoV either in humans or in other animals is not available, [40] but, both the dromedary and human strains of MERS-CoV have comparable replication (in Vero-E6 cells) and respiratory tropism, and both disrupt the interferon responses. [26, 41] Both strains cause alveolar epithelial damage and apoptosis in various parts of the lungs. [42, 43] In transgenic mouse model MERS viral particles were observed in lung, brain, spleen, intestine and heart tissues. [44] Further analysis is necessary to understand the virus-host interaction and the detailed pathogenesis of MERS-CoV infection in *in vivo*.

EPIDEMIOLOGY OF MERS-CoV

Since its first discovery, MERS-CoV has reached more than twenty countries. As of March 2015, most of the MERS-CoV cases had been reported in Saudi Arabia (n=938), followed by the United Arab Emirates, Jordan and Qatar. A handful of cases have been reported from Oman, Egypt, France, Germany, Tunisia, Italy, Algeria, Iran, the Netherlands, Greece, Kuwait, Lebanon, Malaysia, Philippines, Yemen, Austria, Turkey, United Kingdom and United States (Figure 1). [45-49]

Although the progression of the disease in the initial phase was much slower, with the basic reproduction number (R_0) of 0.69 (95% CI 0.50-0.92); the latest estimate suggests that the disease actually has a higher but variable R_0 (ranging from 2 to 6.7) depending on the geographic region (Table 1). [50, 51]

Available data suggest that healthcare exposure is the most important risk factor for MERS-CoV infection. [52-54] Mathematical modelling suggests that nosocomial transmission is over four times higher than community transmission. [55] Other risk factors like low humidity and high temperature are also important, [56] and limited studies suggested a seasonal pattern with spikes in March -April. [57]

CLINICAL FEATURES

Men aged over 45 years, people with pre-existing medical conditions and health care workers are the high risk groups for MERS-CoV infection. [54] The median incubation period for human-to-human transmission is approximately 5 days (range 2–15 days). [9]

MERS-CoV causes symptoms similar to that of SARS but with a distinct clinical course and a high case fatality rate of 35% to 50%. [58, 59] Most cases present with symptoms of

influenza-like illness (ILI) such as fever, cough (predominantly dry), malaise, myalgia, sore throat, headache, rhinorrhoea, nausea, vomiting, abdominal pain, diarrhoea, and even renal failure occur occasionally. [53, 57, 60] Dyspnoea is a frequent complaint, and the majority of the patients develop pneumonia (70%) and ultimately require admission into an intensive care unit (ICU). Concomitant infections and hypoalbuminemia were identified as the predictors of severe infection in individuals aged >65 years. [53] Other extra-pulmonary organ dysfunction such as circulatory collapse, abnormal liver functions and hematological derangements are common in critically ill patients. [61] A second-trimester stillbirth has been reported in a pregnant woman with MERS-CoV. [62]

While MERS-CoV is common in adults; a dozen paediatric cases, including a fatality, have been reported to date. [63] Most of the paediatric cases were asymptomatic and found during the screening process among close family contacts of MERS-CoV patients in the community or in hospital. Children with underlying medical conditions are at higher risk of MERS-CoV infection. [63] Mortality from MERS-CoV is higher in men and in those with pre-existing medical conditions. [7] The median time from symptom onset to hospitalisation is approximately 4 days, and time to ICU admission is approximately 5 days, and ventilatory care is required for a median of 16 days. The duration of an ICU stay is about 30 days however fatality occurs at a median of 12 days following symptom onset. The 90 day mortality for MERS patients in ICU is 58%. [9]

The chest radiograph of a MERS-CoV patient typically shows bilateral enhanced hilar vascular shadows (more prominent on the left side), accentuated bronchovascular markings with multiple patchy opacities in the middle and lower lung fields. Consolidation of the right upper lobe can occur as early as one day after the onset of illness and ground glass opacities and

consolidation of the left lower lobe can occur within 4 of symptom onset, bilateral ground-glass opacities and consolidation and can occur respectively 7 and 9 days after the onset of illness. [4, 64]

DIAGNOSIS OF MERS-COV

Since most MERS-CoV cases present with symptoms similar to other respiratory viral infections, case detection purely based on syndromic diagnosis is challenging. Since the first discovery of the virus, comprehensive laboratory testings have been developed. [56, 65]

Serological assays have been widely used to detect MERS-CoV antibody in dromedary camels. [56, 66, 67] Conventional and rapid biological safe immunofluorescence assays to detect MERS-CoV antibodies have been published. [68] IgG and IgM antibodies in serum samples could be determined using an anti-MERS-CoV indirect immunofluorescence assay. [56, 67, 69]

In addition, the enzyme-linked immunosorbent assays (ELISA), protein microarray technology and micro-neutralisation (MN) assays have also been developed, and have high sensitivity and specificity to detect MERS-CoV antibodies. [56, 67, 70] For seroepidemiological studies, assays like pseudoparticle virus neutralisation test (ppNT) and a conventional MN assay could be used to detect antibodies to MERS-CoV. [49, 71] Western blotting is also useful in serological diagnosis of MERS-CoV. [71, 72] However, serological tests may lack validity and cross react with other coronaviruses. [56]

According to the World Health Organization (WHO) and experts in the field, the screening RT-PCR targeting Up E gene should be conducted on samples from suspected MERS patients. All positive samples should undergo confirmatory testing by targeting *ORF 1a*, *ORF 1b* or *N* gene. [73] Endeavours should be made to obtain lower respiratory tract samples such as

bronchoalveolar lavage and tracheal aspirate, since the viral loads and genome fractions are higher in lower respiratory tract samples. [63] Sequencing data can be used to construct a phylogenetic tree in order to measure the genetic distance between the viruses of different intermediate hosts. [66, 74]

MANAGEMENT OF MERS-CoV

To date, no approved antiviral therapy or vaccination is available for MERS-CoV. [58, 75] Based on *in vitro* experiments or experience from SARS patients various treatment options have been attempted or suggested. [39, 76] Omrani et al. (2014) showed that in patients with severe MERS-CoV infection, ribavirin and interferon alfa-2a therapy are significantly associated with improved survival at 14 days, but not at 28 days. [58] *In vitro* studies also suggest that the ribavirin and interferon alpha-2b combination therapy has significant antiviral effects. [77]

Using distinct clones of anti-CD26 monoclonal antibodies, the domains of CD26 involved in the binding of MERS-CoV have been identified. [78] It has been suggested that 2F9, a clone of CD26, and YS110, a humanised monoclonal antibody against CD26, could be potential therapeutic agents for MERS-CoV. [78] One of the antibodies, m336, neutralises the virus with exceptional potency, and therefore, has potential as a candidate drug and could be even useful in vaccine design. [79] Additionally, human MicroRNAs might be useful as antiviral therapy against MERS-CoV infection. [80] In case of hospitalised patients, the untested convalescent-phase plasma has been suggested as a supportive therapy to minimise the severity of infection. [81]

A few candidate vaccines have been tested in mice with some promising results. [82] Subunit vaccines based on MERS-CoV spike protein and its RBD could be useful in the development of MERS-CoV vaccine. [83, 84] Inhibiting papain-like or 3C-like proteases of MERS-CoV which regulate the polyproteins in MERS-CoV genomic RNA could be a useful concept in vaccine design. [85]

The control of MERS-CoV primarily relies on case-based surveillance; early diagnosis is warranted when infection is suspected. [86] The role of respiratory protective equipment such as surgical mask and N95 respirators have been discussed but not yet proven. [87] A large study is currently underway that is examining the role of facemasks against MERS-CoV among Hajj pilgrims. [88]

Although person to person transmission is limited, travellers to the Middle East could be at risk of exposure to MERS-CoV. [89-92] Raising awareness of MERS-CoV transmission is important in view of the fact that many travellers are not aware of the MERS-CoV outbreak. [93]

It is essential to intensify infection control measures in health care settings, particularly through health education and awareness. [94] Health-care workers also need to follow stringent precautions while handling suspected MERS-CoV patients including using eye protectors and other personal protective equipments. [55] Immunocompromised individuals and those with pre-existing medical conditions should avoid close contact with dromedary camels particularly if the virus is known to be circulating in an area. Similarly raw camel milk, meat and urine should be avoided.

CURRENT RESEARCH GAPS

An important current research gap is that the mechanism by which most people acquired MERS-CoV is unclear; the mechanism of exposure (whether direct or indirect) is difficult to explain and the experiments are fraught with challenges. [47, 95] Serological and molecular evidence of the presence of the virus have been established in domestic dromedaries [96-98] but not in other livestock. [99, 100] The virus has also been recovered from respiratory, gastrointestinal and other bodily secretions or samples of dromedary camels and fruit bats, [66, 96, 101, 102] but serological evidence of MERS-CoV in animal workers is rare. [103] MERS-CoV has been detected from air samples collected from a barn that sheltered an infected camel owned by an infected patient indicating possible airborne transmission of the virus. [104]

Secondly, the direction of transmission, whether from humans to camels or vice versa is unknown. MERS-CoV was identified in dromedary camels in a Qatari barn, which was linked to two confirmed human cases who have since recovered, [66] and it was also possible to inoculate MERS-CoV into healthy camels ultimately producing upper respiratory tract symptoms, [105] but it could not be established whether the people on the farm are infected by the camels or vice versa, or if a third source was responsible. [60]

Thirdly, it remains unclear if a third [intermediate] host is playing a key role in the transmission chain. Phylogenetic studies have revealed a close relationship between MERS-CoV in humans and coronaviruses in bats but the exact virus has not been confirmed in bats. [21, 106] It is noteworthy that most other human coronaviruses have emerged upon transmission from bats to other animal species. [13, 104] Although others reported that a short genomic sequence isolated from an Egyptian tomb bat (*Taphozous perforates*) was identical to

that of EMC/2012 MERS-CoV Essen isolate (KC875821); there is a dearth of information establishing association with the bat virus. [60]

Fourthly, a few reports suggested that MERS-CoV can transmit from human-to- human, [107-109] but, the available data show uncertainty or only limited human-to-human transmission of the virus. [30, 55, 108, 110-114] Low levels of virus shedding might be an explanation why human-to-human transmission is limited or unlikely. [66, 115]

Finally, the evolutionary background of MERS-CoV is still unclear. [116] MERS-CoV might have been circulating in camels in Saudi Arabia since at least 1992. [100] MERS-CoV antibody was found in African camels during 1992-2013 suggesting that the virus has existed in camels for long time. [117, 118] However, MERS-CoV in camels may have undergone a mutation several years ago, allowing the virus to infect humans.

FUTURE RESEARCH DIRECTIONS

- There is a paucity of data describing the transmission cycle of MERS-CoV in various hosts. Well-designed large scale case-control studies are needed to define the transmission chain of MERS-CoV.
- Research into safe and effective antiviral treatments need to be prioritised for a condition with a high mortality rate that threatens as a pandemic.

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CONFLICTS OF INTEREST

None.

Legends

Figure 1. Geographical distribution of confirmed MERS-CoV cases

Table 1. Contrast between SARS and MERS-CoV in respect to their virology, epidemiology and clinical outcomes (inset the number of cases and deaths in the Middle East)

TABLE 1

	MERS-CoV	SARS
Virology	<i>Betacoronavirus</i> lineage 2C	<i>Betacoronavirus</i> lineage 2B
Receptor	hDPP4	ACE2
Genome size	29.9 kb	29.3kb
Source	Not yet confirmed, camel is the likely host	Civet cat
Epidemiology	Limited human to human transmission, the disease is mostly localised in the Middle East	Human to human transmission is recognised, affected many countries but spared the Middle East
Cases (as of 9 th March, 2015)	~1082 (deaths 439)	~8098 (deaths 774)
R ₀	2-3 (for Jeddah 3.5-6.7, for Riyadh 2-2.8)	Variable ranges from 2-6
Superspreading events	Not known	Reported
M:F	1.74:1	0.75:1
Median age (range) in years	48 (1-99)	40 (1-91)
Mean incubation period in days	5.2 (2-15)	4 (2-14)

Comorbidities	Three quarter of the patients had comorbidities	Less than a third had Comorbidities
Clinical presentation	Unpredictable and erratic clinical course ranging from asymptomatic illness to severe pneumonia	A typical biphasic clinical course
Haemoptysis	More common	Less common
Respiratory failure	Presents relatively early	Presents relatively late
Travel association	Limited travel-associated exposure	Recognised travel-associated exposure
Time from symptom onset to hospitalisation	0-16 days	2-8 days
Median time from symptom onset to death	12 days	21 days

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